Briefing Document
Biological Response Modifiers Advisory Committee
Meeting #36
Allogeneic Pancreatic Islets for Type 1 Diabetes
October 9-10, 2003

Contents

INTRODUCTION

Allogeneic pancreatic islet transplantation is a field of intense clinical research as a potential therapy for patients with type 1 diabetes mellitus (DM). This research typically involves the administration of allogeneic islets, derived from cadaveric organs, into the recipient's portal vein. Published preliminary data suggest, in the short term, the safety risks of administering the final product may be reasonable considering the appearance of some clinical benefit. However, a number of manufacturing and clinical issues remain to be addressed in order to obtain product and clinical data sufficient to successfully support a biologics license application (BLA).

MEETING GOALS

This meeting of the Biological Response Modifiers Advisory Committee (BRMAC) was organized to achieve the following goals:

- ?? For FDA to discuss expectations for manufacturing data and clinical evidence to be provided in a BLA for marketing approval of allogeneic islets as a treatment for type 1 diabetes mellitus.
- ?? To obtain perspectives and advice from members of the BRMAC regarding the type and quality of manufacturing, preclinical and clinical data to be provided within a BLA for the marketing approval of allogeneic pancreatic islets as biological products.
- ?? To provide a public forum to obtain input from stakeholders interested in this therapy.

BACKGROUND

During the 10-year period from 1990 to 2000, the Food and Drug Administration (FDA) received a total of 10 investigational new drug applications (INDs) to investigate the use of allogeneic islets as a therapy for type 1 diabetes mellitus. A BRMAC meeting was held in March 2000 in order to identify, clarify, and resolve certain issues relating to exploratory clinical studies for allogeneic islets. The issues discussed at that meeting primarily related to information on organ procurement practices, islet processing techniques, islet characterization procedures, pre-clinical studies to support clinical studies, and early clinical development plans that should be provided in an IND.

In September 2000, FDA mailed a "Dear Colleague" letter to over 250 transplant centers in the United States reminding interested parties that allogeneic islets are regulated as biological products and prior to the initiation of clinical studies sponsors must submit an IND to FDA (Attachment 1).

The input received from the members of the BRMAC, its guest advisors, and the public proved to be timely in light of a July 2000 report in the *New England Journal of Medicine* (NEJM) describing the treatment of seven diabetic patients with allogeneic islets isolated from cadavers (Attachment 2). In this study, subjects received multiple

administrations of allogeneic islets and a steroid-free immunosuppressive regimen. The median follow-up for these seven patients, at the time of the NEJM publication, was 11.9 months (range 4.4 to 14.9 months). The apparent success was remarkable given prior reports of little evidence of clinical benefit from allogeneic islet administration. For example, the International Islet Transplantation Registry reported that, for the ten years between 1989-1999, of the 267 patients who had received allogeneic islets, only 12% were insulin independent for ? 7 days (Attachment 3).

The findings reported in the July 2000 NEJM ("the Edmonton Protocol") resulted in a large increase in the number of centers performing clinical studies using allogeneic islets. In the nearly 4 years (2000-03) since the BRMAC meeting, FDA has received an additional 28 INDs for allogeneic islets. In these INDs, sponsors generally seek to replicate and/or extend the results of the Edmonton Protocol.

FDA has worked with the islet transplant community and other federal agencies to facilitate clinical studies of allogeneic islets. These federal agencies include the Health Resources and Services Administration (HRSA) and the National Institutes of Health (NIH). This collaboration has helped to identify and resolve obstacles to product development in this research area. Other outreach efforts have included participation in at least 10 workshops or conferences related to allogeneic islets as well as publication of an article describing FDA's IND review expectations for allogeneic islets (Attachment 4). We have made substantial outreach efforts in order to help guide interested stakeholders toward the shared goal of collecting the necessary data to demonstrate both the safety and efficacy of these products.

BIOLOGICS LICENSE APPLICATIONS FOR ALLOGENEIC ISLET PRODUCTS

Upon submission of adequate data to demonstrate safety and clinical efficacy, as well as other assurances that a product "meets standards designed to assure that the biological product continues to be safe, pure, and potent, "[?] a biologics license (BLA) may be issued. Thus, clinical development programs using allogeneic islets for treatment of type 1 DM must design their studies to obtain data demonstrating:

- ?? The manufacturing process and facility are sufficient to assure the safety, purity, and potency of the product.
- ?? Substantial clinical evidence of the product's safety and effectiveness.

Manufacturing Quality and Control

Cellular biological products are complex mixtures of multiple components, prepared from living sources. Because these products are difficult to characterize, the manufacturing process helps to define the composition of the final product.

[?] Public Health Service Act, Title 42, USC §262, §351.

Manufacturing changes can impact product safety, identity, purity, potency, consistency and stability in unforeseen ways. The manufacturing process must be sufficient to prevent the transmission or introduction of infectious agents while preserving product identity, purity and potency. The integrated system of documented manufacturing control, in-process testing and final product release testing must be assessed to ensure the final product is safe, pure, and potent for patient administration.

Although most allogeneic islet manufacturing procedures are similar, there is no standard procedure. Some of the variation in isolating islets is a result of the variability in the organ; different organs may require modified processing parameters to optimize the yield and quality of islets. Some of the variation is a result of different preferences and facilities; procedures and equipment vary from center to center. During investigational studies (IND) a certain amount of processing innovation is expected and encouraged. However, for a BLA it is expected that a well-defined manufacturing protocol be established and followed to ensure manufacturing consistency and product quality. It is expected that the manufacturing process and the manufacturing facility will be compliant with current good manufacturing practices (cGMP), meeting lot release testing requirements such as safety, identity, purity, potency (as described in 21 CFR 610– General Biological Products Standards). Further, since islets are not amenable to terminal sterilization, the facilities should be designed for aseptic processing, meeting at least the minimum standards as described in 21 CFR 211.42-211.58. The flow of the manufacturing process should be designed to minimize chances for cross contamination and product mix-up. In-process tests should be performed at critical points in the manufacturing process with appropriate acceptance limits established during the validation process.

So in addition to evaluating the safety and efficacy data submitted in a BLA, FDA will evaluate the manufacturing data to determine if there is a well-established islet preparation process and a track record of product manufacturing consistency; confirm that the islets are prepared in a manufacturing facility meeting cGMP requirements; and verify compliance with applicable lot release testing requirements.

Ensuring Quality of Source Pancreata

Unlike most drugs or other biologics, the source material for allogeneic islets, being cadaveric organs, cannot be controlled in a traditional way. Thus, each donor pancreas has a unique set of characteristics (ischemia time, organ size, age, extent of fibrosis, etc). FDA recognizes that organ procurement and allocation is clearly outside its jurisdiction, nevertheless it is clear that consistency of islet manufacturing is highly dependent on the quality of the organ delivered to the manufacturing facility. In light of this constraint and in order to be compliant with cGMPs and biologics licensing requirements, it is imperative that predefined acceptance criteria for assessing organ quality be established, standardized and validated to the extent possible to ensure that unsuitable organs are excluded.

CMC Question:

1. A key component for ensuring control of a validated islet manufacturing process is the use of pre-defined acceptance criteria to ensure that suitable donor organs with maximal potential for yielding adequate numbers of islets are utilized for manufacturing allogeneic islets, while unsuitable organs are excluded. Acceptance criteria may include donor suitability determination, organ characteristics, harvesting conditions, and transport conditions. Most, if not all, of this information is currently collected by sponsors of islet INDs. Please discuss the use of this manufacturing experience data as a basis for developing pre-defined acceptance criteria for source organs.

Manufacturing of Islets

One consequence of having limited control over the source donor pancreata used to prepare islets is the need to allow some flexibility in the exact procedures used for a specific pancreas in order to optimize the yield and quality of islets. FDA has reviewed various manufacturing procedures that attempt to improve islet number and quality, such as:

- Modifying the organ transport media to include an oxygen-enriched layer of perfluorocarbon
- Varying quantity or units of dissociation enzymes used to reflect specific organ characteristics such as organ size, age, extent of fibrosis, etc.
- Including certain additives (DNAse, Pefabloc, etc.) during islet processing and isolation
- Using short-term culture before islet administration.

FDA agrees flexibility is permissible if conducted using predefined criteria or algorithms, based upon data from manufacturing experience, that establish processing controls within the context of a validated manufacturing protocol.

CMC Question:

2. Based on a given donor organ's characteristics, investigators frequently "customize" an islet isolation procedure by using different reagents, reagent concentrations and processing methods such that no two islet isolation procedures are exactly alike. However, cGMPs and BLA requirements necessitate the use of a well-defined, well-controlled manufacturing process. Is it reasonable to expect that criteria or algorithms can be developed, based on data collected during IND studies, to predetermine under what conditions the use of a specific reagent, reagent concentration, or processing method is appropriate?

Assessing the Final Product

FDA regulations for biological products mandate that specific product safety and quality testing be done on the final product, with results available prior to patient administration. As mentioned above, the product testing standards are prescribed in 21 CFR 610 and include manufacturing safety (sterility, endotoxin, mycoplasma [if cultured]), identity, purity, and potency. When feasible, additional safety testing and other assessments are also performed throughout manufacturing (in-process testing) in order to evaluate the manufacturing process itself and to ensure the quality and consistency of the product lots.

Of the prescribed assays, potency presents a unique challenge. In general, assays for product potency are intended to show the ability of the product to effect a given result. Currently, assays for islet product potency, such as glucose stimulated insulin release or injection of islets under the kidney capsule of diabetic mice, are performed retrospectively. However, prior to the approval of a BLA some reasonable measure of product potency must be developed such that results are available prior to patient administration.

CMC Question:

3. Please discuss any assay or assays that are currently, or could be, performed on the final islet product before patient administration, which may be predictive of the ability of the islets to perform as expected after patient administration.

Islet Comparability

As mentioned previously, the manufacturing process helps to define the product. Manufacturing changes can impact product safety, identity, purity, potency, consistency, and stability in unforeseen ways. Demonstrating that critical product characteristics have not changed even when the process has changed is referred to as comparability. Comparability testing may include analytical assays, bioassays, preclinical studies, and clinical studies. Until comparability can be determined, products manufactured using different processes are considered to be different products.

At present, it is unclear how differences in methods to prepare allogeneic islets by various groups impact the characteristics of the final allogeneic islet product. On an individual basis, many of these manufacturing changes appear to be minor, such as including additives (protease inhibitors, DNAse, etc) in the dissociation medium; dissociating allogeneic islet using manual vs. semi-automated equipment; using a refrigerated or non-refrigerated COBE cell processor unit for allogeneic islet enrichment; or using fresh allogeneic islets vs. using allogeneic islets after short term culture. However, the overall effect of any or all of these changes is not known.

FDA foresees the potential for more than one allogeneic islet sponsor to "pool" their clinical data to support a BLA. While this can be an acceptable approach it is highly dependent on demonstrating comparability. Without sufficient data to demonstrate that allogeneic islets prepared using different methods are comparable, it may not be possible

for FDA to accept data in a license application that is "pooled" from allogeneic islet preparations in which the manufacturing process used was different.

CMC Question:

4. What should be key criteria for demonstrating allogeneic islet product comparability? Please discuss appropriate analytical assays, bioassays, preclinical studies, and clinical studies that may be required.

Preclinical Considerations

Preclinical (pharmacology/toxicology) considerations for licensure of allogeneic islets are not fundamentally different from those applied to other biological therapeutic products. The types of preclinical *in vitro* and *in vivo* studies employing various animal models that should be performed as product development proceeds are reflective of FDA's science-based paradigm of biologics regulation.

When properly designed, performed, and evaluated, preclinical studies can generate data that contribute to the design of scientifically sound clinical development programs. The goals of preclinical safety studies are several-fold: 1) to provide supportive data for an initial safe starting dose and subsequent dose escalation scheme; 2) to aid in determining a risk/benefit assessment for the proposed clinical studies; 3) to identify potential endpoints for detection of toxicity and the clinical monitoring for those toxicities; and 4) to guide in designing appropriate patient eligibility criteria.

Animal Models of Testing

In order to generate scientifically valid data for use in safety, as well as efficacy assessments, it is essential that the cellular product (clinical product or species-specific analogous cells) being tested is biologically active in the animal species used in the preclinical studies. Although traditionally, many *in vivo* safety studies are preferentially performed in healthy animals, clinical investigations of allogeneic islets have been supported by studies in a wide range of animal species, e.g., mouse, rat, dog, pig, monkey and baboon. Many animal models of diabetes have also been studied, including those with genetic mutations (i.e., inbred NOD mice), medication/toxin-induced (i.e., streptozocin treatment, corticosteroid treatment) and surgical (i.e., pancreatectomy) disease. Each of these models has inherent strengths and weaknesses, thus no single model will be completely predictive of the clinical scenario. However, the data generated from these models provide a strong scientific rationale for the potential efficacy of allogeneic islets, thus serving to justify clinical studies in humans with type 1 DM [1]. Importantly, the collection of preclinical safety and efficacy endpoint data can be achieved by applying principles of toxicological study design to these already established animal models of diabetes. These studies should be done according to good laboratory practices (GLP) or be as GLP-compliant (with an emphasis on data integrity and quality) as possible, given the scientific and practical restraints that may be imposed due to the nature of the diabetes model.

Preclinical Evaluation of Immunosuppressive Regimens

A primary focus of the March 2000 BRMAC meeting was the performance of additional preclinical studies for the various immunosuppressive agents proposed for clinical use. From the presentations and ensuing discussions, it was concluded that insufficient preclinical data existed to justify the use of any particular immunosuppressive regimen in diabetic patients receiving allogeneic islets. General agreement was reached on the point that, "…justification of any given immunosuppressive or tolerance-inducing strategy should be based on multiple preclinical models including, but not limited to, specific islet transplantation models and at least some nonhuman primate work" [1]. In concordance with the committee recommendations, FDA advises each IND sponsor to submit preclinical toxicology data in appropriate animal model(s), that are intended to support the safety of the short-term and long-term use of each individual immunosuppressive agent used, as well as any combination of agents, prior to initiation of a clinical trial proposing use of the respective regimen. In addition, FDA advises each IND sponsor anticipating use of a particular immunosuppressive regimen in late stage clinical trials (Phase 3), that the potential for reproductive/ developmental and carcinogenic toxicity of the respective immunosuppressive regimen needs to be addressed in preclinical studies.

Preclinical Assessment of Innovative Routes of Administration

Several IND sponsors currently studying allogeneic islets have proposed that alteration of the route of administration of the cellular product from the widely utilized route of percutaneous transhepatic portal vein delivery to another route (such as a transjugular approach or intraoperative administration) may promote improved islet function, as well as a better overall safety profile. However, as concluded in the March 2000 BRMAC meeting, sponsors proposing innovative alternate delivery methods for allogeneic islets need to demonstrate an adequate safety profile in animals prior to proceeding to clinical trials [1].

Reproductive, Developmental and Carcinogenic Potential of the Cell Product

Based on the current knowledge regarding cellular products, the level of concern regarding reproductive, developmental toxicity, and carcinogenic potential raised by the administration of allogeneic islet products that are collected, isolated, processed, and characterized according to current FDA-specified requirements does not appear to require preclinical studies to assess these potential toxicities. This conclusion is based on the existing extensive literature in the public domain for islet transplantation in many different animal models of varying ages. Knowledge of the biology of these cells does not provide scientific evidence for a plausible mechanism for these products to pose reproductive/developmental or carcinogenic risks to humans at the present time.

Potential Future Issues

It is important, however, to emphasize that future alterations to the production of the allogeneic islets that result in deviation from an accepted/licensed product could significantly change the risks associated with the cellular product. For example, alteration of the islets by gene transfer, or efforts at increasing the proliferative potential of the cells by any post-harvesting manipulation would alter FDA's level of concern for the potential

risks to humans significantly enough to require additional preclinical pharmacology/ toxicology testing prior to the initiation of clinical trials using the new cellular product.

Clinical Data

The clinical review processes for allogeneic islet IND are the same as those for investigational biologic or drug therapies. Consequently, the clinical development programs for allogeneic islets are expected to mirror the paradigm for development of biologic or drug therapies. This regulatory paradigm has been previously summarized (see Weber, et. al).

Islet Study Background

Human cadaveric islet transplantation has been primarily used in patients with type 1 DM, a disease which results from a selective autoimmune destruction of insulin-producing beta (?) cells in the pancreas. Patients with type 1 DM require replacement therapy with exogenous insulin in order to prevent acute and chronic complications. The most common acute complications include various hyperglycemia and hypoglycemic events while the most common chronic complications include nephropathy, neuropathy, retinopathy, and vasculopathy.

In a subgroup of patients with type 1 DM, maintenance of near-to-normal blood glucose levels by intensive exogenous administration of insulin may result in the increased occurrence of episodes of life-threatening hypoglycemia. Additionally, some patients receiving intensive exogenous insulin therapy still develop the chronic complications of diabetes mellitus.

Transplantation of insulin-producing tissues has proven, when successful, to be capable of maintaining excellent metabolic control in the absence of life-threatening hypoglycemia. However, whole-organ pancreas transplantation, while allowing for long-term glucose metabolic control, insulin independence in the absence of hypoglycemic episodes, and reversal of diabetic complications, is often associated with significant morbidity and mortality. Islet tissue administration, on the other hand, presents the advantage of a relatively rapid and somewhat less invasive procedure of implantation; islets are generally injected into the liver by percutaneous transhepatic catheterization of the portal vein, a procedure associated with relatively few perioperative risks and morbidites. The procedure has, at times, been performed in type 1 diabetic patients with end-stage renal failure, as a simultaneous islet-kidney (SIK) or as an islet-after kidney (IAK) transplant. Islet transplantation is also sometimes performed after whole pancreatic graft failure, since whole-organ pancreas re-transplants are associated with an elevated rate of graft failure. Islet autotransplantation has also been performed in patients undergoing pancreatectomy for “benign” disease, including insulin-requiring diabetes mellitus due to cystic fibrosis and hemochromatosis. Islet transplantation for type 2 DM has been attempted in very few cases.

Historically, islet transplantation has been considered an experimental procedure characterized by marginal success, and has been regarded as a therapeutic option

primarily when combined other organ transplant e.g., SIK or IAK. This use was thought to justify the use of chronic immunosuppression.

The immunosuppressive protocols utilized in past years included monoclonal or polyclonal T-cell antibodies such as ALG, ATG, OKT3, or anti-CD4 -based induction immunosuppression, and triple-drug maintenance therapy based on glucocorticoids, cyclosporine or tacrolimus, and azathioprine or mycophenolate mofetil. Unfortunately, most of the immunosuppressive drugs such as glucocorticoids and calcineurin inhibitors are known to exert toxic effects on the islet ? cells or to induce insulin resistance.

The anti-rejection protocols commonly used for solid organ transplantation may have detrimental consequences on islet graft fate. The International Islet Transplant Registry reported that a total of 405 adult islet allogeneic transplants were performed from 1983 through 1998 (see White, et. al.). Analysis of 200 C-peptide negative type 1 diabetic patients transplanted from 1990 through 1997 showed a cumulative 1-year patient survival of 96%, and graft survival assessed by measurable basal C-peptide levels above 0.5 ng/ml of 35%, with insulin independence at 1 year in only 8% of the recipients. The rate of insulin independence was higher in patients receiving multiple-donor islet preparations (19%), when compared to those receiving single-donor preparations (11%), most likely reflecting the need for a conspicuous islet graft mass higher than 6,000 islet equivalents per kilogram (IE/kg) of the recipient's body weight.

The results reported by Shapiro et al. in the NEJM in July 2000 on the successful series of cadaveric human islet cell transplants in type 1 diabetic patients treated with a glucocorticoid-free immunosuppression protocol, including a short course of anti-IL2 receptor antibody, and maintenance therapy based on rapamycin and low dose of tacrolimus, has been thought to represent a major breakthrough in the field of islet transplantation. In this study, all seven patients received islets isolated from more than one donor. Two to three grafts were generally needed in order to obtain an islet mass sufficient to achieve insulin independence (11,547 ? 1,604, mean ? SD, IE/kg of recipient's body weight). Although infusion of islets from a single donor did not result in insulin independence in any of the patients studied, all the patients had improved glycemic control, reduced insulin requirements, and absence of hypoglycemic episodes soon after receiving the first graft. Two-year follow-up data has shown continued glycemic control in a number of these subjects (Attachment 5). A possible explanation of the need for multiple donors in this study is the destruction of a substantial mass of islets immediately after transplantation. Early loss of implanted islets has been proposed to be due to their susceptibility to microenvironment alteration with consequent functional impairment and loss to apoptosis.

Despite the apparent successful results of the Edmonton protocol, the need to administer islets isolated from more than one organ per recipient to achieve insulin independence represents a major hurdle to be solved due to the shortage of organs. The need for chronic immunosuppression also limits the application of allogeneic islet therapy to the small cohort of patients for whom the risks of chronic immunosuppression are outweighed by the potential benefits of islet transplantation.

Historically, immunosuppressive regimens have frequently been modified according to the availability of new molecules. Several strategies are at present under evaluation, which in general aim to improve graft acceptance and/or induce tolerance. In more than 20 of the IND currently in effect, the immunosuppressive regimen being used consists of sirolimus, tacrolimus and daclizumab. The use of OKT3 ?1 (Ala-Ala), etanercept, and a few other immunomodulatory strategies are being pursued under other INDs.

Factors Influencing Islet Graft Function

Several factors are known to contribute to allogeneic islet engraftment and function. The number of islets implanted appears to be a critical factor in obtaining complete function. This number depends upon the condition of the organ donor, the procurement and preservation of the pancreas, and the procedure and reagents used during isolation and purification. All these variables influence yield and quality, purity and viability of the graft, making it difficult to completely standardize the procedures.

Early after transplantation, islets are denervated, not yet vascularized, implanted into a new microenvironment in the absence of extracellular matrix, and are exposed to nonspecific inflammatory events occurring at the site of implant. These are all conditions which may result in functional impairment. After engraftment, islets are exposed not only to allorecognition and rejection, but also to recurrence of the autoimmunity that causes type 1 DM. Furthermore, immunosuppressive drugs might also exert some toxicity on islet ? cells, impairing their function.

Outcome Measures in the Clinical Studies

Glycemic control

The extent of mean fluctuations in serum glucose concentrations, measured as mean amplitude of glycemic variation in the 24hr period is a useful tool in the assessment of metabolic instability. The results obtained with the 'Edmonton protocol' showed relatively dramatic improvement following sequential islet cell transplants, with decreased overall mean serum glucose concentrations, mean amplitude of glycemic variations, and lability of glycemic control in a 24hr period.

Glycosylated hemoglobin

Hemoglobin A1c (HbA1c) is formed from the irreversible nonenzymatic glycation of the hemoglobin ? chain, and is directly proportional to the ambient glucose concentration. The level of HbA1c highly correlates with blood sugar levels and lasts longer after the maximum blood sugar level is observed, making it a more reliable long-term marker of blood sugar level control than immediate glycemia measurement. The value of HbA1c as a retrospective and cumulative marker of glycemic balance in diabetic patients has been confirmed by epidemiological studies. In the case of intensive insulin therapy, the desired end result is a glycemic level slightly higher than normal, to prevent hypoglycemic episodes. In the long-term, however, this might result in delay, and not prevention, of the occurrence of chronic secondary complications of diabetes. Successful

allogeneic islet administration has been reported to improve the glucose metabolic control with return of HbA1c levels within the normal range. Also, in long-term functioning whole pancreas transplants, the achievement of improved glucose metabolic control has been associated with normalization of HbA1c during the first 2 years after transplantation, and normal or near-normal levels over a 6-year period, in conjunction with small doses of exogenous insulin and in the absence of hypoglycemic episodes. However, because of the cumulative nature of this marker, it may not represent a good means of monitoring short-term alteration in islet function and, therefore, it is not a valuable early marker of graft rejection/autoimmune recurrence.

Insulin requirements

Insulin requirement inversely correlates with glucose metabolic control. One of the goals of successful allogeneic islet administration is the achievement of a status of normoglycemia and insulin independence, in the absence of insulin resistance. In the data of the Edmonton group presented in the NEJM, all seven subjects maintained insulin independence at 1-year follow-up post-transplant. While inversely correlated with the mass of functionally competent islets, insulin requirement does not represent a viable early rejection recurrence marker.

C-peptide levels

Allogeneic islet administration is considered partially successful when C-peptide levels (? 0.3 ng/ml) are detectable in patients who were C-peptide negative, pre-transplant. C-peptide and insulin are produced in equimolar amounts from the proinsulin molecule by pancreatic ? cells, and measurement of plasma C-peptide allows monitoring of ? cell function when a patient is treated with exogenous insulin.

Persistent C-peptide secretion several years after transplant in type 1 diabetic recipients has been correlated with long-term normalization or near-normalization of blood glucose control, and significant improvement of HbA1c levels in the absence of severe episodes of hypoglycemia.

Mixed meal tolerance test

Mixed meal tolerance tests are utilized to assess islet ? cell function in response to stimuli; in these studies, basal and peak insulin C-peptide levels are expressed as an index. The mixed meal tolerance test (MMTT) consists of the administration of 6 kcal/kg of Sustacal 140 gr/l carbohydrates, 24 g/l fat, and 61 g/l protein; 1 kcal/ml and calculation of the stimulation index between basal and peak C-peptide levels.

When mixed meal tolerance tests are performed in patients with only one kidney SIK or IAK, it is important to consider that C-peptide half-life is prolonged by about 40%, rendering the interpretation of C-peptide as measurement of insulin production difficult.

In patients in whom insulin independence has been achieved after islet cell transplant, MMTT might reveal impairment of function. Although a viable means of monitoring islet function, MMTT is not performed with high frequency, limiting the possibility of early detection of graft impairment.

Intravenous glucose tolerance test

It has been reported that first-phase insulin release in response to intravenous glucose tolerance test (IVGTT) provides an accurate reflection of islet ? cell mass, and its use is valuable in the long-term monitoring of the islet graft mass after transplant. It consists of the infusion of glucose (0.3g/kg body weight), over 1 min and collection of serial samples of arterial blood before and after injection for C-peptide determination. A variation of the test has been used with additional ? cell stimulation with tolbutamide (300 mg) 20 minutes later and the stimulation index of insulin secretion is calculated. Similar to the oral GTT, this assay cannot be performed frequently and in a manner that represents a simple and viable marker of early graft failure.

Monitoring of autoimmunity

Standardized markers for monitoring the autoimmune response to transplanted islets are not available to date. Analyses of humoral autoimmune responses, such as monitoring of autoantibodies titers, have been proposed. A correlation between rising titers of antibodies directed at glutamate decarboxylase, GAD 65, and tyrosine phosphatase, IA-2, and graft loss due to recurrence of autoimmunity has been reported in whole-pancreas transplantation. Progressive islet graft failure has been observed earlier in autoantibody-positive than in autoantibody-negative recipients of intrahepatic islet allografts. Further studies are necessary to establish the predictive value of these antibodies for delineating the recurrence of autoimmunity.

Monitoring of islet graft-specific cellular auto- and allo-reactivity in peripheral blood has also been suggested as a valuable tool to better understand the mechanisms influencing islet allograft survival in diabetic patients. In vitro assaying of the proliferative response of T lymphocytes to certain islet autoantigens such as GAD65, insulin, insulin-secretory granules, ICA69, h38kD, r38kD, insulinoma membranes and control stimuli bovine serum albumin, aB-cystallin, and tetanus toxoid has been proposed for the detection of changes in the recipient autoimmune reactivity that might correlate with graft outcome; the predictive value of such parameters to assess cell-mediated autoimmunity recurrence awaits further confirmation.

Monitoring of rejection

Early markers of rejection of islet allografts are not yet available. Many investigators regard imaging analyses and serial tissue biopsies as too cumbersome or uninformative. Often the diagnosis of rejection is done at the occurrence of hyperglycemia, a time point when a conspicuous mass of islet has already been lost. At this time point, attempts at graft rescue through antirejection treatments may be futile.

A correlation between islet allograft failure and increased titers of anti-donor HLA antibodies has been reported (as measured by panel reactive antibody testing). Considerable progress in developing a molecular-based diagnostic approach to define early markers for rejection has recently been made, and quantitative analysis of the genes involved in the cytolytic machinery of cytotoxic T-lymphocytes including granzyme B, perforin, and Fas ligand in the peripheral blood has been proposed as an approach to detect early episodes of rejection and drive antirejection therapy.

Alloreactivity assayed by cytotoxic T lymphocyte precursors and T-helper frequencies against all mismatched HLA antigens of islet donors might allow for the differentiation between the occurrences of auto- or alloreactivity. A similar approach has been reported in experimental islet transplantation to monitor the occurrence of allorejection by performing serial mixed lymphocyte-islet co-culture.

Clinical Questions:

Multiple clinical studies of allogeneic islets are completed or on-going. Some clinical data will be presented or discussed by sponsors during this meeting, but none of these data have undergone FDA review and none of these data should directly impact your responses to FDA's questions. Instead, the presentations of these data are intended to provide examples of the types of data currently being accumulated within the clinical investigative field.

In light of the steady progress in clinical development of allogeneic islet products, FDA is seeking objective and independent advice on certain aspects of clinical data that may be important in designing confirmatory (phase 3) clinical studies and/or in interpreting the meaningfulness of clinical data from other types of clinical studies.

1. Please discuss the clinical importance and limitations of each of the outcomes listed below. Please discuss each outcome with respect to its use in providing substantial evidence of efficacy if adequate and well-controlled clinical studies demonstrate a robust and durable treatment effect on the outcome.

    a. "Insulin independence." This outcome may be defined in many ways but, in addition to any other definitions you regard as important, please specifically comment upon the following definition: "cessation of insulin treatment or decrease in insulin requirement in the setting of sufficient glycemic control." If you regard this as an important endpoint, please comment upon what you regard as evidence of "sufficient glycemic control."

    c. Use of hemoglobin A1C, serum C-peptide concentration and mean amplitude of glycemic excursions (independently or in various composites) as measures of glycemic control and/or islet function.

    d. Acute diabetic complications. Please identify those complications that you regard as important outcomes (e.g., episodes of hypoglycemic unawareness, hospitalizations, death).

    e. Long-term diabetic sequelae. Please identify those complications that you regard as important outcomes (e.g., nephropathy, neuropathy, etc.).

f. Other outcomes you regard as important.

2. Regarding the overall clinical development program for a sponsor's allogeneic islets, please discuss the importance and/or meaningfulness of the following types of clinical data with respect to the ability to form a risk-benefit assessment for the product.

   a. Certain types of safety data: Specifically, the nature and extent of "long-term" clinical data that must be submitted in order to form a reasonable risk-benefit assessment. For example, must clinical follow-up for a certain number of subjects extend over a protracted period of time (e.g., 3, 5 or more years)? If so, please comment upon what you regard as a reasonable period of time and describe the types of data that must be obtained during this period, both pre- and post-licensure.

   b. Historically controlled clinical data: Specifically, the appropriateness of the use of historical controls in the development program for a product, and whether data from studies that use no concurrent controls could be sufficient to provide substantial evidence of effectiveness of the product.

   c. Clinical data from studies that only enrolled subjects with certain specific baseline characteristics: Specifically, the use and potential generalizability of clinical data from studies that enrolled a small subset of subjects with type 1 DM (for example, only subjects with a history of certain manifestations of "hypoglycemic unawareness"). Additionally, please discuss those baseline characteristics that you regard as important for a sponsor to consider in the clinical development of their product (e.g., age, extent and nature of diabetic complications, etc.).

ATTACHMENTS:

1) FDA "Dear Colleague" Letter (http://www.fda.gov/cber/ltr/allpan090800.htm)

2) Shapiro, A, Lakey, J, Ryan, E. Korbutt, G., Toth, E., Warnock, G., Kneteman, N., Rajotte, R. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000; 343:230-8.

3) White, S., James, R., Swift, S., Kimber, R., Nicholson, M. Human islet cell transplantation—future prospects. Diabetic Medicine 2001; 18:78-103.

4) Weber, D., McFarland, R., Irony, I. Selected Food and Drug Administration Review Issues for Regulation of Allogeneic Islets of Langerhans as Somatic Cell Therapy. Transplantation 2002; 74:1816-1820.

5) Ryan, E, Lakey, J., Paty, B., Imes, S., Korbutt, G., Kneteman, N., Bigam, D., Rajotte, R., Shapiro, A. Successful islet transplantation; continued insulin reserve provides long-term glycemic control 2002. Diabetes; 51:2148-2157.

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